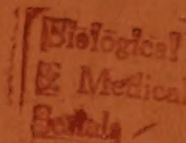


U. S. National Institute of Health
Bulletin No. 2

BULLETIN OF THE HYGIENIC LABORATORY,

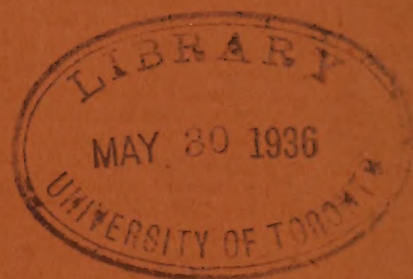
Treasury Department, U. S. Marine-Hospital Service,

WALTER WYMAN, Surgeon-General.



FORMALIN DISINFECTION OF BAGGAGE

WITHOUT APPARATUS.



M. J. ROSENAU,

PASSED ASSISTANT SURGEON, DIRECTOR HYGIENIC LABORATORY,

U. S. MARINE-HOSPITAL SERVICE,

WASHINGTON, D. C.

WASHINGTON :

GOVERNMENT PRINTING OFFICE.

1900.

BULLETIN OF THE HYGIENIC LABORATORY,

Treasury Department, U. S. Marine-Hospital Service,

WALTER WYMAN, Surgeon-General.

FORMALIN DISINFECTION OF BAGGAGE

WITHOUT APPARATUS.

M. J. ROSENAU,

PASSED ASSISTANT SURGEON, DIRECTOR HYGIENIC LABORATORY,

U. S. MARINE-HOSPITAL SERVICE.

WASHINGTON, D. C.

WASHINGTON :

GOVERNMENT PRINTING OFFICE.

1900.

FORMALIN DISINFECTION OF BAGGAGE WITHOUT APPARATUS.

This work was undertaken on account of the rather widespread use of formalin for the disinfection of baggage by sprinkling it on clothing in trunks, or by pouring it on absorbent material in the corners of a trunk. From what follows, it will be seen that the method is not a good one.

Formaldehyd possesses an antiseptic power much stronger than any of the organic nontoxic substances. It has, however, distinct limitations, which must be understood in order to properly apply it to practical disinfection. It is the object of this work to point out one of these limitations.

Formaldehyd (formic aldehyd) is a complex and unstable substance. It exists as a gas, in solution, and as a solid. In solution it is a powerful disinfectant. The gas formaldehyd is a valuable disinfectant, but is so unstable and so difficult to obtain in its gaseous state that its use in unskilled hands may prove unsatisfactory.

Formaldehyd exists in at least three well-recognized isomeric states :

1. Formaldehyd (formic aldehyd) is a gas at ordinary temperatures, colorless, and possessing slight odor, but having an extremely irritating effect upon the mucous membrane of the nose and conjunctiva. At a temperature of about — 20° C. the gas polymerizes into paraformaldehyd, known commercially as paraform.

2. Paraform is a white substance, unctious to the touch, soluble in both water and alcohol. It consists chemically of two molecules of formaldehyd. It is this substance which is supposed to compose the commercial solutions of formaldehyd, known as formalin, formol, etc.

3. Trioxymethylen is formed by the union of three molecules of formaldehyd. It is a white powder giving off a strong odor of the gas. It is but slightly soluble in alcohol and water.

To sum up, formaldehyd exists in three states :

$\text{CH}_2\text{O}.$	$\begin{array}{c} \text{CH}_2\text{O} \\ \\ \text{CH}_2\text{O}. \end{array}$	$\begin{array}{c} \text{CH}_2\text{O} \\ \\ \text{CH}_2\text{O} \\ \\ \text{CH}_2\text{O}. \end{array}$
Formaldehyd gas.	Paraformaldehyd paraform.	Trioxymethylen.

It must be plain to all who have worked with the substance known in commerce as formalin, formol, etc., that it can not be a 40 per cent solution of the gas formaldehyd in water, as claimed. It more likely

consists of a solution of the isomeric forms of formaldehyd, especially paraformaldehyd.

Formalin solutions are unstable. In cold weather they are apt to precipitate a white substance, usually trioxymethylen. Heating and evaporating them has a tendency to change the formaldehyd to paraform.

These facts are stated to emphasize the importance of not considering commercial "formalin" as a simple solution of a gas in water. This has no bearing in using formalin as a liquid disinfectant in the same way that we would use solutions of bichloride of mercury or carbolic acid, but it has a very distinct and important bearing in gaseous disinfection with formaldehyd.

It is not the scope of the present article to dwell upon the best way to obtain the gas formaldehyd from its solution. The present work points out the difficulty of obtaining the gas without special apparatus from its solution in a closed container.

The power of formaldehyd gas as a disinfectant depends on the chemical change which takes place between the gas and the albuminous matter of the germ. To be effective, therefore, the gas must not only be brought in direct contact with the germ, but in sufficient amount and for a sufficient length of time. In this respect it does not differ from bichloride of mercury and other chemical disinfectants. This point is especially mentioned here on account of the difficulty of obtaining formaldehyd gas in its free state, and the uncertainty as to the amount of gas evolved from its solution, important factors which must be taken into account in practical disinfection.

Formaldehyd forms a chemical compound with albuminous matter from which it is impossible to again separate it. It renders gelatin insoluble when added in small amounts. Egg albumen, to which formalin has been added, can not be coagulated by heat. This property of "fixing" organic matter makes it valuable as a re-agent in microscopy and explains some of its limitations as a disinfectant.

Methods.

Young bouillon cultures of typhoid and diphtheria, one to two days old, and older cultures of anthrax, containing spores, were used to impregnate slips of paper.

White filter paper is cut into slips about 1 inch long, by one-fourth of an inch wide and sterilized by dry heat. These are convenient to drop into test tubes. The slips are impregnated with the bouillon cultures and exposed between the blankets and sheets in the boxes.

Freshly prepared, moist slips were used throughout this work, because moist cultures are more readily killed by formaldehyd than dry ones, and it was designed throughout to make the conditions favorable for the disinfecting action of the gas to act.

The blankets and sheets used in these experiments are folded to fit the boxes and then sterilized with steam. The slips of paper contain-

ing the organisms are placed between the fold of the sterile blankets or sheets. The rule was to allow the blankets and sheets to dry. Sometimes they were used while still moist, but when this latter was done it is always specifically noted.

Three methods were adopted in placing the formalin in the boxes: 1, sprinkling; 2, pouring; 3, soaking on absorbent cotton.

Particular pains were taken in sprinkling because it was found that the result depends upon the technique of sprinkling.

The formalin was sprinkled from a pipette, drawn out so as to deliver a small drop. The solution was dropped from the pipette upon the blankets or sheets, as the case may be, spread out on the bottom of the box. The drops were evenly distributed over the surface and sufficiently far apart so as not to run together. It makes considerable difference in the results whether the formalin is poured on one place or carefully sprinkled in the manner indicated.

The time of exposure varied from twenty-four hours to five days, and is stated for each test. The boxes were ordinary pine packing cases, made reasonably gas tight by the use of impervious paper and varnish. They closely imitated the conditions presented by a trunk. In all other respects the experiments were made to imitate as closely as possible the conditions found in packing the average trunk.

After charging the boxes the covers were nailed down and left at the room temperature, which in this laboratory is about 18° to 25° C. When the time of exposure has expired the boxes are opened and the slips of paper are inoculated into bouillon without delay. The large size test tubes, two-thirds full of bouillon, were used in order to dilute the trace of formaldehyd which sometimes remains. The method of neutralizing the slips of paper after exposure to formaldehyd with ammonia before inoculating them into bouillon was not used in all cases, especially in the earlier parts of the work. From and including test No. 39 all the slips were neutralized as follows:

After the exposure the slips are held fifteen to thirty seconds in a beaker containing ammonia and then planted in bouillon. It is well to wash out the formamide formed by the combination of the formaldehyd and ammonia with a little distilled and sterile water, but that this is not necessary is shown in the following test:

Slips of filter paper impregnated with bouillon cultures of typhoid, anthrax, and diphtheria, prepared as described above, were placed in a petri dish and exposed to formaldehyd for ten minutes by placing a little pledget of cotton containing a few drops of formalin in the same dish. Some of the slips were then inoculated into bouillon with the following result:

Typhoid (3 slips), no growth.

Anthrax (3 slips), all grew.

Diphtheria (3 slips), no growth.

The others were exposed to ammonia before inoculation into bouillon, with the following result:

Typhoid (3 slips), all grew.

Anthrax (3 slips), all grew.

Diphtheria (3 slips), all grew.

It will therefore be seen that the formaldehyd inhibited but did not kill the typhoid and diphtheria, for no visible growth was obtained from the slips planted directly from their formaldehyd exposures into bouillon, whereas, those first treated with ammonia showed a good growth.

It is therefore reasonable to suppose that in some instances throughout this work the same phenomenon resulted, especially in the tests in which the trace of formaldehyd was not neutralized with ammonia. This possible error, however, does not effect the conclusions drawn from the work, because the results obtained show that formalin when used in a closed container has feeble powers of penetration and can not be recommended as a safe and effective disinfectant.

If some of the cultures exposed were inhibited and not killed this conclusion would be still more positive.

The bouillon tubes inoculated with the slips of paper are incubated at 37° C. In case a growth does not appear, they are kept at least ten days, frequently two or three weeks, before discarding.

In many instances the growth was found to be retarded. No visible growth before five to seven days.

In case the tubes showed positively the growth was always tested for purity. In few instances contamination crept in, usually a mold from the air or a rod resembling subtilis.

Control slips were always kept under equal conditions for twenty-four hours and inoculated as above to verify every test.

Viability of cultures.

The cultures used in these observations were anthrax, diphtheria, and typhoid. When exposed in bouillon cultures to heat they gave the following results:

Typhoid—

Five minutes at 65° C., growth.

Ten minutes at 65° C., growth.

Fifteen minutes at 65° C., slight growth.

Twenty minutes at 65° C., no growth.

Diphtheria—

Five minutes at 65° C., growth.

Ten minutes at 65° C., growth.

Fifteen minutes at 65° C., no growth.

Twenty minutes at 65° C., no growth.

Anthrax spores—

Two minutes in steam, growth.

Three minutes in steam, growth.

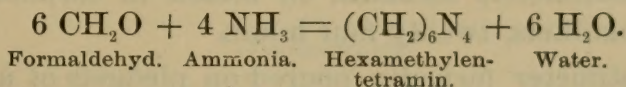
Five minutes in steam, no growth.

Eight minutes in steam, no growth.

Strength of formaldehyd used.

The same formalin was used in all these experiments. Its strength was estimated by the quantitative method given by Trillat in *La Formaldehyde et ses Applications*, 1896, and Ernst J. Lederle, Ph. D., in the *New York Medical Journal* for October 16, 1897, and is briefly as follows:

The principle on which the method is based is that ammonia and formaldehyd combine quite readily to form the compound hexamethylen-tetramin, according to the following reaction:



From the amount of ammonia required to form this compound the formaldehyd is calculated. The best results are obtained when an excess of ammonia is used, the mixture allowed to stand for at least twelve hours, with occasional shaking, and the excess of ammonia present determined by means of sulphuric acid, using coralline (rosalic acid) as an indicator.

Method of analysis.—Take the specific gravity of the solution at the room temperature.

Place 2 or 3 cubic centimeters, carefully measured, into a bottle with a glass stopper; add 50 to 60 cubic centimeters N/2 ammonia solution; shake well and let stand twelve hours, shaking occasionally. Then titrate with N/4 sulphuric acid, using coralline (rosalic acid) as an indicator.

Calculation.—The cubic centimeters of ammonia neutralized by formaldehyd = cubic centimeters of ammonia used, minus cubic centimeters of sulphuric acid.

The per cent strength of the solution =

$$\frac{2.25 \times \text{c. c. ammonia neutralized by formaldehyd.}}{\text{Grammes of solution taken.}}$$

Grammes of solution taken.

In case the solution under examination is acid, the amount is first to be determined by a fixed alkali and a corresponding correction made in the above calculation.

In accordance with this method six estimations were made of the formalin solution used in these experiments, with the following results:

	Per cent.
Test No. 1.....	40. 90
Test No. 2.....	40. 02
Test No. 3.....	40. 20
Test No. 4.....	40. 50
Test No. 5.....	40. 27
Test No. 6.....	39. 60
Average	40. 25

NOTE.—I desire to acknowledge the assistance in the latter part of this work rendered by Asst. Surg. L. D. Fricks, United States Marine-Hospital Service.

POURING THE FORMALIN ON ABSORBENT COTTON.—TIME OF EXPOSURE, TWENTY-FOUR HOURS.

No. 1.

Four cubic centimeters formalin on cotton in corners of box, cultures between blankets, twenty-four hours.

One cubic centimeter formalin poured on pledgets of absorbent cotton placed in each corner of a box 15 by 15 by 15 inches. Four cubic centimeters in all. Moist freshly prepared cultures on slips of filter paper placed between blankets near the center of the box. Box opened after twenty-four hours.

Layers of blankets.	Typhoid.	Anthrax spores.	Diphtheria.
1.....	+	+	+
2.....	—	+	+
3.....	+	+	+
5.....	+	+	+
7.....	+	+	+
Controls.....	+	+	+

No. 2.

One hundred cubic centimeters formalin on cotton in corners of box, cultures between blankets, twenty-four hours.

Twenty-five cubic centimeters of formalin poured on pledgets of absorbent cotton in each corner of a box. One hundred cubic centimeters in all. Size of box 2 by 2 by 2 feet. Fresh moist cultures on slips of filter paper placed between layers of a blanket at various distances from the corners. The blanket was laid at the bottom of the box, smoothed out but not pressed down and no weight above it. Exposure, twenty-four hours.

Layers of blankets.	Anthrax spores.	Diphtheria.
1.....	+	+
4.....	+	+
6.....	+	+
Controls.....	+	+

No. 3.

One hundred cubic centimeters formalin on cotton in corners, cultures between layers of *sheets*, twenty-four hours.

Twenty-five cubic centimeters formalin poured on absorbent cotton tucked into each corner of a box 2 by 2 by 2 feet. One hundred cubic centimeters in all.

The freshly prepared, moist slips of filter paper, impregnated with bouillon cultures placed between layers of sheets. Box opened after twenty-four hours.

Layers of sheets.	Typhoid.	Anthrax spores.	Diph- theria.
2	+	+	+
8	(c) —	(c) —	+
16			+
24			+
Controls	+	+	+

c Contaminated.

No. 4.

Twenty-five cubic centimeters formalin saturated on cotton and suspended in box. Cultures between layers of *sheets*. Twenty-four hours.

Twenty-five cubic centimeters formalin poured on a pledget of absorbent cotton, suspended in a box, 15 by 15 by 15 inches.

Fresh moist cultures on slips of filter paper placed between layers of sheets, laid without pressure at bottom of box. Twenty-four hours' exposure.

Layers of sheets.	Typhoid.	Anthrax spores.	Diph- theria.
9		+	—
10		+	—
17		+	+
41		+	+
57		+	+
73	—	+	+
89	—	+	+
Controls	+	+	+

From the foregoing four experiments (Nos. 1, 2, 3, and 4) it is plain that even large quantities of formalin do not, in twenty-four hours, give off sufficient formaldehyd to penetrate between the layers of blankets or sheets when poured on absorbent material in the corners of, or suspended in a box. The pledgets of absorbent cotton are still moist after twenty-four hours' exposure and the formaldehyd gas has not been liberated from its solution in sufficient quantity to be effective between the layers of a blanket loosely laid on the bottom of the box. Slightly better effect is found with sheets.

The method is, therefore, not applicable for disinfection, with twenty-four hours' exposure.

POURING THE FORMALIN ON ONE SPOT—TIME OF EXPOSURE, TWENTY-FOUR HOURS.

The following five observations, Nos. 5, 6, 7, 8, and 9, were designed to determine to what distance formalin is efficacious when poured on blankets and sheets. The formalin in these five observations was poured in a measured amount on one spot or edge of the blanket or sheet, and the cultures placed at varying distances and separated by varying layers.

In two instances, Nos. 7 and 8, the formalin was poured on a strip of absorbent cotton, laid along the edge of the blanket, instead of pouring it directly on the blanket.

No. 5.

One cubic centimeter poured on one spot, blanket, twenty-four hours.

One cubic centimeter formalin poured on one spot on a blanket at bottom of box 3 feet 4 inches by 1 foot 9 inches by 1 foot 9 inches. Slips of filter paper, moist and freshly prepared from bouillon cultures, placed between layers of blankets 2 feet away from the formalin. Twenty-four hours' exposure.

Layers of blankets.	Typhoid.	Anthrax spores.	Diph- theria.
1	+	+	+
2	+	+	+
3	+	(a)	+
4	+	+	+
5	+	+	+
6	+	+	+
7	+	+	+
Controls	+	+	+

a Lost.

No. 6.

One cubic centimeter poured along the edge, blanket, twenty-four hours.

One cubic centimeter formalin poured along the edge of a blanket. Moist cultures in filter paper 8 inches from formalin and between the layers, as stated in the table. Box opened after twenty-four hours.

Layers of blankets.	Typhoid.	Diph- theria.
1	+	+
2	+	+
3	+	+
4	+	+
5	+	+
Controls	+	+

No. 7.

Two cubic centimeters on absorbent cotton, edge of blanket, twenty-four hours.

Two cubic centimeters formalin on a strip of absorbent cotton placed along the edge of a blanket at one end of box 15 by 15 by 15 inches. Moist cultures at a distance of 8 inches between layers of the same blanket. Box opened in twenty-four hours.

Layers of blankets.	Typhoid.	Diphtheria.
1.....	+	-
2.....	+	-
3.....	-	+
4.....	+	+
Controls.....	+	+

No. 8.

Three cubic centimeters on strip of absorbent cotton, edge of blanket, twenty-four hours.

Three cubic centimeters formalin on a strip of absorbent cotton placed along the edge of a blanket at one end of box, 3 feet 4 inches by 1 foot 9 inches by 1 foot 9 inches.

Fresh moist cultures placed at distances of 8 inches, 12 inches, 18 inches, and 24 inches, between layers of the same blanket.

Box opened after twenty-four hours exposure.

	Layers of blankets.	Inches—			
		8	12	18	24
Typhoid	1	+	+	+	+
Diphtheria.....	1	+	+	+	+
Typhoid	2	+	+	+	+
Diphtheria.....	2	+	+	+	+
Typhoid	3	+	+	+	+
Diphtheria.....	3	+	+	+	+
Typhoid	4	+	+	+	+
Diphtheria.....	4	+	+	+	+
Controls.....		+	+	+	+

No. 9.

Four cubic centimeters poured along the edge of sheet, twenty-four hours.

Four cubic centimeters formalin poured from a pipette on the edge of a *sheet* at bottom of box 15 by 15 by 15 inches, and fresh moist cul-

tures placed at measured distances between the several layers of the same sheet.

	Typhoid.	Anthrax Spores.	Diph- theria.
Fourth layer, 5 inches.....	—	+	—
Do. 12 inches.....	—	+	—
Do. 17 inches.....	+	+	—
Eighth layer, 6 inches.....	—	+	—
Do. 12 inches.....	—	+	—
Controls.....	+	+	+

It is plain from the above that formalin has no penetration through blankets when poured in one place. It seems to be equally ineffectual, whether the formalin is poured directly on the blanket, along its edge, or on absorbent material placed in the corners of, or suspended in a box, in the twenty-four hours' exposure.

A slightly better result is noticed with sheets. Typhoid and diphtheria are killed through 4 and 8 layers at a distance of 12 inches. No effect, however, was obtained with anthrax spores.

The effect of sprinkling the formalin so that the small drops may have better chance to evaporate and liberate the gas was next tried with better results.

SPRINKLING SMALL AMOUNTS OF FORMALIN ON SHEETS AND BLANKETS—TIME OF EXPOSURE, TWENTY-FOUR HOURS.

In the following tests in which the formalin was *sprinkled*—this was carefully done from a pipette drawn out so as to deliver small drops. Pains were taken to distribute the solution in small drops, uniformly, over the surface of the blanket or sheet. The drops were far enough apart so as not to run together.

No. 10.

Twenty-five hundredths cubic centimeter formalin per square foot sprinkled on blanket, twenty-four hours exposure.

One cubic centimeter formalin sprinkled on the surface of a blanket at the bottom of a box 2 by 2 by 2 feet. The cultures on slips of filter paper, fresh and moist, placed between the layers of blankets.

Layers of blankets.	Typhoid.	Anthrax spores.	Diph- theria.
1	—	+	+
2	+	—	—
3	+	+	—
4	+	+	+
5	+	+	+
6	+	+	+
Controls.....	+	+	+

No. 11.

Five-tenths cubic centimeter formalin per square foot, sprinkled, blankets, twenty-four hours' exposure.

Two cubic centimeters formalin sprinkled on a blanket at the bottom of a box 2 by 2 by 2 feet. The cultures on slips of paper, moist and freshly prepared, placed between layers of blankets, exposed twenty four hours.

Layers of blankets.	Typhoid.	Anthrax spores	Diph- theria.
1	+	+	+
2	+	+	+
3	+	+	—
4	+	+	+
5	+	+	—
6	+	+	—
7	+	+	—
8	+	+	—
9	+	+	—
10	(a)		
11	+		
12	+		
13	+		
14	+		
15	+		
16	+		
Controls.....	+	+	+

a Lost.

No. 12.

Five-tenths cubic centimeter per square foot, sprinkled, formalin twenty-four hours.

Three cubic centimeters formalin sprinkled on blanket at bottom of a box 3 feet 4 inches by 1 foot 9 inches by 1 foot 9 inches. Fresh moist cultures on slips of filter paper. Box opened after twenty-four hours.

Layers of blankets.	Typhoid.	Anthrax spores.	Diph- theria.	Remarks.
1.....	—	+	—	a Small growth and retarded. Do.
2.....	+	+	—	
3.....	+	+	(a) +	
4.....	—	+	+	
5.....	+	+	—	
6.....	+	+	+	
7.....	+	+	+	
8.....	+	+	+	
9.....	+	+	+	
10.....	+	+	+	
11.....	+	+	+	
Controls..	+	+	+	

No. 13.

Six-tenths cubic centimeter per square foot, sprinkled on blanket, twenty-four hours' exposure.

One cubic centimeter formalin sprinkled on blanket at bottom of

a box 15 by 15 by 15 inches. Fresh moist cultures on slips of filter paper. Box opened after twenty-four hours' exposure.

Layers of blankets.	Typhoid.	Anthrax spores.	Diphtheria.
1	—	+	—
2	—	+	+
3	—	+	+
4	—	+	+
5	+	+	+
Controls	+	+	—

In the foregoing four experiments, Nos. 10, 11, 12, and 13, the blankets were dry. The small amount of formalin used failed to kill diphtheria through 1 layer. In the following experiment, No. 14, the blankets were moist, the other conditions remaining the same:

No. 14.

Six-tenths cubic centimeter per square foot, *moist* blankets, twenty-four hours.

One cubic centimeter formalin sprinkled on moist blanket at bottom of a box 15 by 15 by 15 inches. Fresh moist cultures on slips of filter paper placed between layers of moist blankets. Box opened after twenty-four hours exposure. Blankets still moist.

Layers of blankets.	Typhoid.	Diphtheria.
1	+	+
2	+	—
3	+	—
4	+	+
5	+	+
Controls	+	+

In this experiment (No. 14) we have an irregular result. Small quantities of formalin produce irregular results in almost all the published works on this subject.

We may, therefore, conclude from the foregoing tests that small quantities of formalin sprinkled on blankets and sheets have practically no disinfecting power in a closed container, excepting on the spot where the solution falls, after twenty-four hours' exposure.

SPRINKLING DILUTE FORMALIN ON BLANKETS AND SHEETS—TIME OF EXPOSURE, TWENTY-FOUR HOURS.

Small quantities of formalin having very little effect the next series of experiments were designed to determine whether small amounts of formalin diluted and well sprinkled would aid the evolution and penetration of the gas. Observations Nos. 15, 16, 17, 18, and 19 were made with 1 c. c. of formalin diluted with 4, 5, 10, 15, and 25 cubic centimeters of water, respectively.

No. 15.

Six-tenths cubic centimeters diluted per square foot, sprinkled on blankets, twenty-four hours.

One cubic centimeter of formalin diluted with 4 c. c. of distilled water sprinkled in small drops on a blanket carefully smoothed out over the bottom of a box 15 by 15 by 15 inches. Freshly prepared cultures on slips of filter paper, still moist, placed between layers of blankets. Box opened after twenty-four hours.

Layers of blankets.	Typhoid.	Anthrax spores.	Diphtheria.
1	--	+	--
2	--	+	--
3	--	+	--
4	--	+	+
6	--	+	+
8	+	+	--
Controls	+	+	+

No. 16.

Six-tenths cubic centimeter formalin diluted per square foot, sprinkled, blankets, twenty-four hours.

One cubic centimeter diluted in 5 c. c. water; otherwise the same conditions as No. 14.

Layers of blankets.	Typhoid.	Anthrax spores.	Diphtheria.
1	--	--	+
2	--	+	+
3	--	+	+
4	--	--	--
5	--	+	--
Controls.....	+	+	+

No. 17.

Six-tenths cubic centimeter formalin diluted per square foot, sprinkled, blankets, twenty-four hours.

One cubic centimeter formalin and 15 c. c. water, sprinkled on blankets; otherwise the same conditions as Nos. 14 and 15.

Layers of blankets.	Typhoid.	Anthrax spores.	Diphtheria.
1	--	--	+
2	--	+	--
3	--	--	--
4	--	(c) --	--
5	--	(c) +	--
Controls	+	+	+

c Contaminated.

No. 18.

Six-tenths cubic centimeter formalin diluted per square foot, sprinkled, blankets, twenty-four hours.

One cubic centimeter formalin and 15 c. c. water; otherwise the same conditions as Nos. 14, 15, and 16.

Layers of blankets.	Typhoid.	Anthrax spores.	Diph- theria.
1	—	+	—
2	(c) —	—	+
3	—	—	—
4	—	+	+
5	(d) —	—	+
Controls	+	+	+

c Contaminated. d Lost.

No. 19.

Six-tenths cubic centimeter formalin diluted per square foot, sprinkled, blankets.

One cubic centimeter formalin, 25 c. c. water; otherwise the same conditions as Nos. 14, 15, 16, and 17.

Layers of blankets.	Typhoid.	Anthrax spores.	Diph- theria.
1	(c) +	—	—
2	+	+	—
3	—	+	+
4	—	+	+
5	—	+	—
Controls	+	+	+

c Contaminated.

Diluting the formalin, therefore, does not increase its efficiency when used by this method—sprinkling. Dilution appears to have somewhat the same influence that moist blankets have, viz, renders its penetration through the blankets very irregular.

Small amounts of formalin used by this method gave such unexpected results that a few more tests were made at a later date to confirm these observations and are inserted here.

No. 20.

Five-tenths cubic centimeter per square foot diluted, sprinkled, blankets.

Two cubic centimeters formalin diluted with 10 c. c. of water sprinkled on a blanket at bottom of a box 2 by 2 by 2 feet.

Fresh moist cultures on slips of filter paper placed between layers of dry blankets. Twenty-four hours' exposure.

Layers of blankets.	Typhoid.	Anthrax spores.	Diphtheria.
1	—	+	—
2	—	+	—
3	—	+	+
4	—	+	—
5	—	+	(a)
Controls	+	+	+

a Lost.

No. 21.

Five-tenths cubic centimeter per square foot, sprinkled, blankets.

Three cubic centimeters formalin diluted with 15 c. c. of distilled water sprinkled on a blanket at bottom of a box 3 feet 4 inches by 1 foot 9 inches by 1 foot 9 inches. Cultures on slips of filter paper, freshly prepared and moist, laid between layers of dry sterilized blankets.

Layers of blankets.	Typhoid.	Anthrax spores.	Diphtheria.
1	—	+	+
2	—	+	+
3	—	+	—
4	—	+	—
5	—	+	+
Controls	+	+	+

Being satisfied that small amounts of formalin used by this method are ineffectual, larger amounts were next tried.

SPRINKLING LARGER AMOUNTS OF FORMALIN ON BLANKETS AND SHEETS.—TIME OF EXPOSURE, TWENTY-FOUR HOURS.

No. 22.

One and three-tenths per square foot, sprinkled, blankets, twenty-four hours.

Two cubic centimeters formalin sprinkled on a blanket at bottom of box 15 by 15 by 15 inches. Cultures on slips of filter paper, freshly prepared, placed between layers of blankets. 'Twenty-four hours' exposure.

Layers of blankets.	Typhoid.	Anthrax spores.	Diphtheria.
1	—	—	(a) +
3	—	+	—
5	+	+	(a) +
7	+	+	+
9	+	+	+
Controls	+	+	+

(a) Retarded.

No. 23.

One and three-tenths cubic centimeters per square foot, sprinkled, blankets, twenty-four hours.

Same conditions as No. 24.

Layers of blankets.	Typhoid.	Anthrax spores.	Diphtheria.
1	—	—	—
2	—	+	—
3	—	+	+
4	—	+	—
5	+	+	+
Controls	+	+	+

The 2 foregoing tests, Nos. 22 and 23, were made with dry blankets. The following one was made with moist blankets; the other conditions being similar:

No. 24.

One and three tenths cubic centimeters per square foot, sprinkled *moist* blankets, twenty-four hours.

Two cubic centimeters formalin sprinkled on a moist blanket at bottom of box 15 by 15 by 15 inches. Cultures fresh and moist as above and placed between the layers of a moist blanket. Box opened after twenty-four hours' exposure. The blankets are still moist.

Layers of blankets.	Typhoid.	Anthrax spores.	Diphtheria.
1	+	+	—
2	(c) +	+	—
3	—	+	+
4	—	+	+
5	—	+	+
Controls	+	+	+

c Contaminated.

It is again evident that the presence of moisture interferes with the penetration of the formaldehyd when used by this method and causes an irregular effect.

In the following test layer amounts, 2 c. c. per square foot were used:

No. 25.

Two cubic centimeters formalin per square foot, sprinkled, blankets, twenty-four hours.

Three cubic centimeters formalin sprinkled on a blanket at bottom of a box 15 by 15 by 15 inches. Cultures fresh and moist on slips of filter

paper placed between layers of blankets. Box opened after twenty-four hours.

Layers of blankets.	Typhoid.	Diph- theria.
1.....	—	—
2.....	—	—
3.....	—	—
4.....	—	—
5.....	—	+
6.....	+	—
8.....	+	—
Controls.....	—	+

No. 26.

Two cubic centimeters formalin per square foot, sprinkled, blankets, twenty-four hours.

Three cubic centimeters formalin sprinkled on a blanket at bottom of a box 15 by 15 by 15 inches. Cultures on slips of paper, freshly prepared and moist, between layers of blankets. Twenty-four hours' exposure.

Layers of blankets.	Typhoid.	Anthrax spores.	Diph- theria.
1.....	—	+	—
2.....	—	(c)	—
3.....	—	+	—
4.....	—	+	—
5.....	(c)	+	—
6.....	(d)	+	+
Controls.....	+	+	+

c Contaminated. d Lost.

No. 27.

Two cubic centimeters per square foot, sprinkled, blankets, twenty-four hours.

Three cubic centimeters formalin sprinkled on a blanket at bottom of a box 15 by 15 by 15 inches. Fresh bouillon cultures of the organism placed on slips of filter paper, twenty-four hours' exposure.

Layers of blankets.	Typhoid.	Diph- theria.
1.....	—	—
2.....	—	—
3.....	—	—
4.....	—	—
5.....	—	—
Controls.....	—	+

It is seen from Nos. 25, 26, and 27, that 2 c. c. of formalin per square foot, when well sprinkled, may be depended upon to penetrate through four layers of blankets and kill typhoid and diphtheria cultures, but it

has no effect at all upon anthrax spores, though separated by only a single layer.

The next tests were made to determine whether diluting the larger amounts of formalin before sprinkling it increased its power of penetration.

LARGER AMOUNTS OF FORMALIN DILUTED AND SPRINKLED.—TIME OF EXPOSURE, TWENTY-FOUR HOURS.

No. 28.

Two cubic centimeters diluted per square foot, sprinkled, blankets, twenty-four hours.

Three cubic centimeters formalin diluted with 5 c. c. of water sprinkled on a blanket on the bottom of a box 15 by 15 by 15 inches. Fresh moist cultures on slips of filter paper placed between layers of blankets. Box opened after twenty-four hours' exposure.

Layers of blankets.	Typhoid.	Diphtheria.
1.....	—	—
2.....	—	—
3.....	—	—
4.....	—	—
5.....	—	—
Controls.....	+	+

No. 29.

Two cubic centimeters formalin diluted per square foot, sprinkled, blankets, twenty-four hours.

Three cubic centimeters formalin diluted with 10 c. c. of water; other conditions the same as No. 28.

Layers of blankets.	Typhoid.	Diphtheria.
1.....	—	—
2.....	—	—
3.....	—	—
4.....	—	—
5.....	—	—
Controls.....	+	+

No. 30.

Two cubic centimeters diluted per square foot, sprinkled, blankets, twenty-four hours.

Three cubic centimeters diluted with 15 c. c. of water; other conditions same as Nos. 28 and 29.

Layers of blankets.	Anthrax spores.	Diphtheria.
1.....	—	—
2.....	—	—
3.....	—	—
4.....	—	—
5.....	+	—
Controls.....	+	+

From Nos. 28, 29, and 30, it appears that 2 c. c. of formalin diluted with 5, 10, and 15 c. c. of water will kill nonspore-bearing organisms, such as typhoid and diphtheria, through 4 layers of blanket, the same as when undiluted.

STILL LARGER AMOUNTS OF FORMALIN, SPRINKLED.—TIME OF EXPOSURE, TWENTY-FOUR HOURS.

No. 31.

Two and seven-tenths cubic centimeters per square foot, sprinkled, blankets, twenty-four hours.

Four cubic centimeters formalin sprinkled on blanket laid in the bottom of a box 15 by 15 by 15 inches. Slips of filter paper impregnated with the cultures, placed while fresh and moist between the blankets. Box opened after twenty-four hours' exposure.

Layers of blankets.	Typhoid.	Anthrax spores.	Diphtheria.
2.....	—	+	—
4.....	—	+	—
6.....	—	+	+
8.....	—	+	+
10.....	—	+	+
12.....	—	+	+
Controls.....	+	+	+

No. 32.

Two and seven-tenths cubic centimeters per square foot, sprinkled, blankets, twenty-four hours.

Four cubic centimeters formalin very carefully sprinkled upon blanket at bottom of a box 15 by 15 by 15 inches. Slips of filter paper

impregnated with the cultures placed while still moist between the layers of blankets.

Layers of blankets.	Typhoid.	Anthrax spores.	Diphtheria.
2	—	—	—
4	—	—	—
6	+	+	—
8	+	+	—
10	+	+	—
Controls	+	+	+

No. 33.

Two and five-tenths cubic centimeters per square foot, sprinkled on blankets, twenty-six hours.

Ten cubic centimeters formalin sprinkled on blanket at bottom of a box 2 by 2 by 2 feet. Cultures on slips of filter paper, moist and freshly prepared from bouillon. Box opened after twenty-six hours.

Layers of blankets.	Typhoid.	Anthrax spores.	Diphtheria.
1	—	—	
2	+	(c)	
3	—	—	—
4	—	+	—
5	—	+	—
6	—	+	—
7			—
8			+
Controls	+	+	+

The effects of 2 and 2.7 c. c. formalin per square foot being quite constant through 4 layers of blankets, and often penetrating through 5 and 8 layers for nonspore-bearing forms of organisms, another trial was now made to contrast the effect of using the same amount of formalin and keeping all the other conditions constant, except pouring the formalin on the blanket instead of sprinkling.

Special attention is called to experiment No. 33. In this test a growth was obtained from typhoid through 2 layers of blanket. Such irregularities occasionally creeping in make an element of uncertainty that renders this method of disinfection very questionable. A good method of disinfection must have no exceptions. It must invariably kill.

ANOTHER TEST BY POURING INSTEAD OF SPRINKLING.—TIME OF EXPOSURE, TWENTY-FOUR HOURS.

No. 34.

Two and seven-tenths cubic centimeters per square foot, blankets, poured, not sprinkled, twenty-four hours.

Four cubic centimeters formalin, poured, not sprinkled, on a blanket laid on the bottom of a box 15 by 15 by 15 inches.

Diphtheria and typhoid cultures from 36-hours-old bouillon growths. Anthrax from six days, containing spores.

Slips of filter paper impregnated with cultures and placed fresh and moist between the layers of sterilized blankets. Box opened after twenty-four hours.

Layers of blankets.	Typhoid.	Anthrax spores.	Diphtheria.
2	—	—	—
4	+	+	—
6	—	+	—
8	+	+	+
Controls	+	+	—

This experiment again emphasizes the importance of sprinkling the formalin in small drops; otherwise the effect is irregular and uncertain.

CULTURES IN COAT POCKET.

No. 35.

Two and seven-tenths per square foot, cultures in coat pocket, twenty-four hours.

Four cubic centimeters formalin sprinkled on a blanket laid on bottom of a box 15 by 15 by 15 inches. The cultures wrapped in sterile paper and placed in the pocket of a coat which was folded and packed in the box so that the cultures were in the inside.

Nature of experiment.	Typhoid.	Anthrax spores.	Diphtheria.
Cultures in coat pocket.....	—	—	—
Do.....	—	+	—
Do.....	—	+	—
Controls.....	+	—	—

Experiment No. 35 gives expected result with nonsporulating organism, but had no effect on anthrax.

FORMALIN SPRINKLED FOR DOWNWARD EFFECT.—TIME OF EXPOSURE,
TWENTY-FOUR HOURS.

No. 36.

Two and seven-tenths cubic centimeters per square foot, blankets, downward effect, twenty-four hours.

All the foregoing observations having been made by sprinkling the formalin on blankets or sheets at the bottom of the box, and the cultures placed between other blankets or sheets laid over this, this observation was made with these conditions reversed—that is, the cultures were placed between the layers of blanket at the bottom of the box and 4 c. c. formalin sprinkled above. About the same effect was obtained.

Layers of blankets.	Typhoid.	Anthrax spores.	Diph- theria.
2	—	—	—
4	+	+	—
6	+	+	—
8	+	+	+
10	+	+	+
Controls	+	+	+

SPRINKLING LARGE AMOUNTS.—TWENTY-FOUR HOURS' EXPOSURE.

No. 37.

Four cubic centimeters per square foot, sprinkled, blankets, twenty-four hours.

Six cubic centimeters formalin sprinkled on blanket at bottom of box 15 by 15 by 15 inches. Cultures fresh and moist placed between layers of blankets as in the other tests.

Layers of blankets.	Typhoid.	Diph- theria.
2.....	—	—
4.....	—	—
6.....	+	—
8.....	+	—
10.....	+	—
Controls.....	+	+

No. 38.

Four and two-tenths cubic centimeters per square foot, sprinkled on blankets, twenty-four hours.

Twenty-five cubic centimeters formalin sprinkled on a blanket in bottom of a box 3 feet 4 inches by 1 foot 9 inches by 1 foot 9 inches. Cul-

tures on slips of filter paper, moist and freshly prepared. Opened after twenty-four hours.

Layers of blankets.	Typhoid.	Anthrax spores.	Diph- theria.
1	—	—	—
2	—	—	(a)
3	—	—	—
4	—	—	—
5	—	+	—
6	—	+	+
7	—	(a)	—
8	—	+	—
9	—	+	—
10	—	+	—
Controls	+	+	+

a Lost.

It appears that increasing the amount of formalin does not correspondingly increase the power of penetration. Four cubic centimeters per square foot is not more effective than 2.5 c. c. per square foot in twenty-four hours' exposure, although more constant in its results.

The next series of experiments were made to determine whether increasing the time would increase the efficiency by this method.

From test No. 39 all the slips were neutralized with ammonia after exposure and before planting in bouillon.

INCREASING THE TIME OF EXPOSURE FROM TWENTY-FOUR HOURS TO FIVE DAYS, ETC.—FORMALIN ON ABSORBENT COTTON IN CORNERS OF BOX.

No. 39.

Ten cubic centimeters formalin in corners of box. Cultures between layers of blanket, five days.

Two and one-half cubic centimeters formalin poured on pledgets of absorbent cotton in each corner of a box 15 by 15 by 15 inches. Ten cubic centimeters in all. Slips of filter paper, moist and freshly prepared from bouillon cultures, were placed between layers of a dry blanket laid on the bottom of the box without pressure.

On opening, after an exposure of five days, the cotton was found to be dry and contained a deposit of paraform.

Layers of blankets.	Typhoid.	Anthrax spores.	Diph- theria.
2	+	+	+
4	+	+	+
6	+	+	+
8	+	+	+
Controls	+	+	+

No. 40.

Fifteen cubic centimeters formalin in corners of box. Cultures between layers of blankets, five days.

Three and one-half cubic centimeters formalin poured on pledgets of absorbent cotton in each corner of a box 15 by 15 by 15 inches. Fifteen cubic centimeters in all. Slips of filter paper moist and freshly prepared from bouillon cultures were placed between layers of a dry blanket laid on the bottom of the box without pressure.

On opening, after an exposure of five days, the cotton was found to be dry and contained a deposit of paraform.

Layers of blankets.	Typhoid.	Anthrax spores.	Diphtheria.
2	+	+	—
4	+	+	—
6	+	+	+
8	+	+	+
Controls	+	+	+

No. 41.

Twenty-five cubic centimeters formalin in corners of box. Cultures between layers of blanket, five days.

Six and one-fourth cubic centimeters formalin poured on pledgets of absorbent cotton in each corner of a box 15 by 15 by 15 inches. Twenty-five cubic centimeters in all. Slips of filter paper moist and freshly prepared from bouillon culture were placed between layers of a dry blanket laid on the bottom of the box without pressure.

On opening, after an exposure of five days, the cotton was found to be dry and contained a deposit of paraform.

Layers of blankets.	Typhoid.	Anthrax spores.	Diphtheria.
2	+	+	+
4	+	+	+
6	+	+	+
8	+	+	+
Controls	+	+	+

From Nos. 39, 40, and 41, it is plain that even five days' exposure, with the formalin in cotton in the corners of the box has no effect. The formaldehyd was deposited as paraform and not enough of the gas was liberated to destroy typhoid and diphtheria. This method is therefore totally inapplicable.

Further trials were then conducted by the method of sprinkling, but increasing the time of exposure to two, three, four, and five days.

FORMALIN SPRINKLED ON BLANKET.—TIME OF EXPOSURE, TWO TO FIVE DAYS.

No. 42.

One and three-tenths cubic centimeters formalin per square foot. Cultures between layers of blankets, seventy-two hours.

Two cubic centimeters formalin sprinkled on a blanket at the bottom of a box 15 by 15 by 15 inches. Slips of filter paper, moist and freshly prepared from bouillon cultures, were placed between layers of dry blankets, seventy-two hours' exposure.

Layers of blankets.	Typhoid.	Anthrax spores.	Diphtheria.
2	—	(a) +	—
4	—	—	—
6	—	—	—
8	+	—	—
Controls	+	—	—

(a) Light growth and retarded.

No. 43.

One and three-tenths cubic centimeters formalin per square foot. Cultures between layers of blankets, ninety hours.

Two cubic centimeters formalin sprinkled on a blanket at the bottom of a box 15 by 15 by 15 inches.

Slips of filter paper moist and freshly prepared from bouillon cultures were placed between layers of dry blankets, ninety hours' exposure.

Layers of blankets.	Typhoid.	Anthrax spores.	Diphtheria.
2	—	—	—
4	—	—	—
6	—	—	—
8	—	—	—
Controls	+	+	+

Experiments Nos. 42 and 45 plainly show that small amounts of formalin sprinkled on blankets and exposed seventy-two and ninety hours do not kill anthrax spores through 2 layers of blanket nor diphtheria through 4 layers.

Larger amounts of formalin were next used.

No. 44.

Two and seven-tenths cubic centimeters formalin per square foot. Cultures between layers of blanket, forty-eight hours.

Four cubic centimeters formalin sprinkled on a blanket at the bottom of a box 15 by 15 by 15 inches.

Slips of filter paper moist and freshly prepared from bouillon cultures were placed between layers of dry blankets, forty-eight hours' exposure.

Layers of blankets.	Typhoid.	Anthrax spores.	Diphtheria.
2	—	—	—
4	—	(a)+	—
6	—	+	—
8	—	+	—
Controls	+	+	+

a Retarded five days—very light growth.

No. 45.

Two and seven-tenths cubic centimeters formalin per square foot. Cultures between layers of blanket, seventy-two hours.

Four cubic centimeters formalin sprinkled on a blanket at the bottom of a box 15 by 15 by 15 inches.

Slips of filter paper moist and freshly prepared from bouillon cultures were placed between layers of dry blankets, seventy-two hours' exposure.

Layers of blankets.	Typhoid.	Anthrax spores.	Diphtheria.
2	—	—	—
4	—	+	+
6	—	+	+
8	+	+	+
Controls	+	+	+

No. 46.

Two and seven-tenths cubic centimeters formalin per square foot. Cultures between layers of blankets, ninety hours.

Four cubic centimeters formalin sprinkled on a blanket at the bottom of a box 15 by 15 by 15 inches.

Slips of filter paper, moist and freshly prepared from bouillon cultures, were placed between layers of dry blankets, ninety hours' exposure.

Layers of blankets.	Typhoid.	Anthrax spores.	Diphtheria.
2	—	—	—
4	—	+	—
6	—	+	+
8	—	+	+
Controls	+	+	+

No. 47.

Two and seven-tenths cubic centimeters formalin per square foot. Cultures between layers of blanket, ninety hours.

Four cubic centimeters formalin sprinkled on a blanket at the bottom of a box 15 by 15 by 15 inches.

Slips of filter paper, moist and freshly prepared from bouillon cultures, were placed between layers of dry blankets, ninety hours' exposure.

Layers of blankets.	Typhoid.	Anthrax spores.	Diphtheria.
2	—	(a) +	—
4	—	—	—
6	—	—	—
8	—	+	+
Controls	+	—	+

(a) Light growth.

From the foregoing four tests, Nos. 44 to 47, it will be seen that 2.7 c. c. of formalin per square foot will not always penetrate through 2 layers of blankets and, never through more than 4 for anthrax spores, and for nonspore-bearing organisms it can not be depended upon through more than 3 layers.

It is therefore concluded that increasing the time of exposure does not increase the power of penetration through blankets.

However, to make the work complete still larger amounts of formalin were used as follows :

LARGE AMOUNTS OF FORMALIN SPRINKLED ON BLANKETS.—TIME OF EXPOSURE, NINETY HOURS.

No. 48.

Four cubic centimeters formalin per square foot. Cultures between layers of blankets, ninety hours.

Six cubic centimeters formalin sprinkled on a blanket at the bottom of a box 15 by 15 by 15 inches. Slips of filter paper moist and freshly prepared from bouillon cultures were placed between layers of dry blankets, ninety hours' exposure.

Layers of blankets.	Typhoid.	Anthrax spores	Diphtheria.
2	—	—	—
4	—	—	—
6	—	+	—
8	—	+	+
Controls	+	—	—

No. 49.

Four cubic centimeters formalin per square foot. Cultures between layers of blanket, ninety hours.

Six cubic centimeters formalin sprinkled on a blanket at the bottom of a box 15 by 15 by 15 inches.

Slips of filter paper moist and freshly prepared from bouillon cultures were placed between layers of dry blankets, ninety hours' exposure.

Layers of blankets.	Typhoid.	Anthrax spores.	Diphtheria.
2	—	—	—
4	—	—	—
6	—	+	+
8	+	+	+
10	+	+	+
Controls	+	+	+

No. 50.

Five cubic centimeters formalin per square foot. Cultures between layers of blanket, ninety hours.

Eight cubic centimeters formalin sprinkled on a blanket at bottom of a box 15 by 15 by 15 inches.

Slips of filter paper moist and freshly prepared from bouillon cultures were placed between layers of dry blankets, ninety hours' exposure.

Layers of blankets.	Typhoid.	Anthrax spores.	Diphtheria.
2	—	—	—
6	—	(a) +	—
8	—	+	—
10	+	+	—
12	+	+	+
Controls	+	+	+

a Small growth.

From Nos. 48, 49 and 50 more constant results are obtained with 4 and 5 c. c. formalin per square foot with ninety hours' exposure than with 2.5 c. c. per square foot.

Cultures were always killed through 4 layers of blankets but not through 6.

It is therefore plain that increasing the time of exposure with this method over twenty-four hours does not correspondingly increase its efficiency.

A few further experiments were made to test its penetration through cotton fabrics with the following results:

SPRINKLED ON SHEETS, MUSLIN, AND CHEESE CLOTH.—TIME OF EXPOSURE, TWO TO THREE DAYS.

No. 51.

Two and seven-tenths cubic centimeters formalin per square foot, sprinkled, sheets, forty-eight hours.

Four cubic centimeters formalin sprinkled on sheet at bottom of a box 15 by 15 by 15 inches. Cultures fresh and moist on slips of filter paper placed between layers of sheet. Box opened after forty-eight hours, culture held in ammonia gas one half minute and then planted in bouillon.

Layers of sheets.	Typhoid.	Anthrax.	Diphtheria.
2	—	—	—
4	—	—	—
18	—	+	—
20	—	+	—
22	—	+	+
24	—	+	+
26	—	+	+
28	—	+	+
Controls	+	+	+

From No. 51 it is seen that formalin will penetrate through about 15 layers of sheets, which, however, does not equal in bulk 3 layers of blankets.

No. 52.

Two and seven-tenths cubic centimeters formalin per square foot, sprinkled, cheese cloth, seventy-two hours.

Four cubic centimeters formalin sprinkled on cheese cloth at bottom of a box 15 by 15 by 15 inches. Cultures freshly prepared and moist on slips of filter paper placed between the layers of the cheese cloth. Box opened after seventy-two hours, cultures held in ammonia gas one-half minute and then planted in bouillon.

Layers of cloth.	Typhoid.	Diphtheria.
26	—	—
38	+	+
51	—	+
64	+	+
78	+	+
88	+	+
Controls	+	+

No. 53.

One and twenty-five hundredths cubic centimeters per square foot, sprinkled, moist *sheets*, forty-eight hours.

Five cubic centimeters formalin sprinkled on sheet at bottom of a box 2 by 2 by 2 feet. Cultures on slips of filter paper, moist. Sheets still moist from sterilizer, forty-eight hours' exposure.

Layers of sheets.	Typhoid.	Anthrax spores.	Diphtheria.
9	—	—	—
10	—	—	—
11	—	—	—
12	—	—	—
14	—	—	—
15	—	—	—
16	—	—	—
17	—	—	—
18	—	—	—
19	—	—	—
Controls	+	+	+

No. 54.

Four cubic centimeters per square foot, sprinkled, cheese cloth, seventy-two hours.

Six cubic centimeters formalin sprinkled on a sheet at bottom of a box 15 by 15 by 15 inches. Fresh moist cultures on slips of filter paper placed between layers of sheet, three days' exposure.

Layers.	Typhoid.	Diphtheria.	Subtilis spores.
20	—	—	—
40	—	—	—
60	—	—	+
80	—	—	+
Controls	+	+	+

No. 55.

Two and seven-tenths cubic centimeters per square foot, sprinkled, sheets, seventy-two hours.

Four cubic centimeters formalin sprinkled on a sheet on bottom of a box 15 by 15 by 15 inches. Fresh moist cultures on slips of filter paper placed between layers of sheet, three days' exposure.

Layers.	Typhoid.	Diphtheria.	Subtilis spores.
8	—	—	+
15	—	—	+
Controls	+	+	+

No. 56.

Two and seven-tenths centimeters per square foot, sprinkled, muslin, seventy-two hours.

Four cubic centimeters formalin sprinkled on sheet at bottom of a box 15 by 15 by 15 inches. Fresh moist cultures on slips of filter paper placed between layers of muslin, three days' exposure.

Layers.	Typhoid.	Diph- theria.	Subtilis spores.
10	+	—	+
20	+	—	+
30	+	—	+
40	+	—	+
50	+	+	+
Controls	+	+	+

CONCLUSIONS.

Large quantities of formalin poured on absorbent material in the corners of or suspended in a box have practically no disinfecting power between layers of blankets or sheets, even after five days' exposure.

The formalin neither evaporates nor liberates its formaldehyd in a closed container at ordinary temperatures (18 to 25° C.) in twenty-four hours.

In two to five days the fluid will evaporate, but the formaldehyd is deposited as paraform on the spot where it was poured.

This method of pouring the formalin on absorbent material is therefore totally inapplicable to the disinfection of baggage, in a closed container.

Sprinkling formalin on baggage in a trunk or box may be depended upon to kill nonspore-bearing organisms, such as typhoid and diphtheria, through 3 layers of blankets.

The method, however, is not a reliable one unless a sufficient quantity of formalin is sprinkled in small drops uniformly over the surface and between many layers.

It can not be depended upon to kill anthrax spores through 1 layer of blanket.

Formaldehyd is given off from its aqueous solution in very small amounts at ordinary temperatures.

The evaporation of the solution in a closed container is slow and uncertain.

In using this method, therefore, great care must be exercised to sprinkle the formalin uniformly, and in small drops. It is best to keep the container in a warm place to facilitate the evolution of the gas.

Use not less than 4 c. c. of formalin, containing 40 per cent formaldehyd, over every square foot of surface between every third layer of

blankets or their equivalent. Somewhat less will suffice for linen and cotton goods.

This means the use of from 50 to 100 c. c. per cubic foot.

Increasing the amount from 2.5 c. c. to 4 c. c. per square foot does not correspondingly increase its power of penetration, but gives constant results.

Increasing the time of exposure over twenty-four hours does not increase the penetrating power of the disinfectant.

Pouring the liquid in one place, and splashing the one liquid on with brushes will not suffice. The formalin must be carefully sprinkled in small drops and distributed uniformly over the surface, between many layers.

Excessive moisture of the fabrics interferes with penetration and gives irregular results.

Formalin, as sold commercially, has an acid reaction. This is sometimes excessive and ruins colors and fabrics. Pure formalin spots and "fixes" leather, and injures certain dyes. Even pure water will spot some delicate silks and colors. Care must therefore be exercised as to the articles with which the formalin comes in contact.

As far as known, all our quarantinable diseases are caused by non-spore-bearing organisms, and our object is to disinfect, not sterilize. However, the quarantine regulations of the United States require the use of chemical and physical agents sufficient to destroy spores. This excess is justified as an element of safety to secure good results in practical work on a large scale. This method of disinfection by sprinkling or pouring formalin in a closed box, as here set forth, does not reach this standard, for even in large amounts it sometimes fails to kill anthrax spores through one layer of blanket, and therefore can not be considered a good method of disinfection.

